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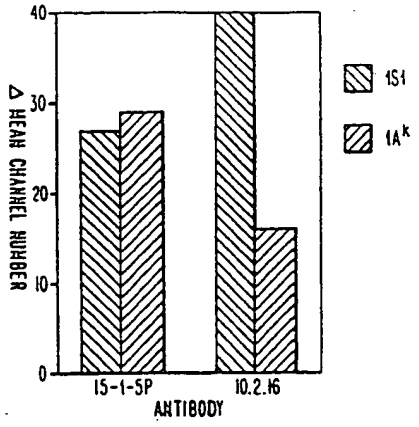
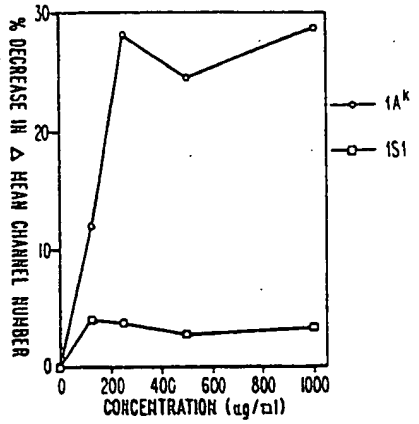
(54) Title: METHOD OF MODULATING MAMMALIAN T-CELL RESPONSE

(57) Abstract

Methods of modulating mammalian T-cell response restricted by an MHC and methods of treating an MHC-linked disease in a mammal suspected of requiring such modulation or treatment, are provided by the invention. The methods comprise treating the mammal or contacting the T-cells respectively with an effective amount of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen.





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METHOD OF MODULATING MAMMALIAN T-CELL RESPONSE

FIELD OF THE INVENTION

This invention relates to the field of mammalian therapeutics. More particularly, the invention relates to novel methods of modulating mammalian T-cell response restricted by an MHC and methods of treating MHC-linked diseases in a mammal with compounds capable of binding with a T-cell antigen receptor that recognize the MHC bound to an antigen.

GOVERNMENT GRANTS

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BACKGROUND OF THE INVENTION

A contributing factor to MHC-linked diseases in mammals, such as rheumatoid arthritis and juvenile diabetes mellitus, is encoded in a portion of chromosome 6 known as the major histocompatibility complex (MHC).

This complex, denoted HLA in the human (Human Leukocyte Antigen), has been divided into five major gene loci, which according to World Health Organization nomenclature are designated HLA-A, HLA-B, HLA-C, HLA-D, and HLA-DR. The A, B, and C loci are single gene loci. The D and DR loci are multi-gene loci. The A, B and C loci encode the classical transplantation antigens, whereas the D and DR loci

encode products that control immune responsiveness. More recent definitions divide the gene products of the HLA loci into three classes (I, II, and III) based on structure and function. Class I encompasses the products of the HLA-A, HLA-B, and HLA-C loci and the Qa/TL region. The products of the HLA-D and HLA-DR related genes fall in Class II. The Class II antigens are believed to be heterodimers composed of an alpha (approx. 34,000 daltons) glycopeptide and a beta (approx. 29,000 daltons) glycopeptide. The number of loci and the gene order of Class II are tentative. The third class, Class III, includes components of complement. As used herein, the term "MHC" is intended to include the above described loci as well as loci that are closely linked thereto.

The class II antigen products are essential in the normal immune response for the triggering of the activation steps which lead to immunity. Even when the immune system is activated inappropriately, and attacks normal tissue, causing autoimmunity, these class II molecules play an essential role in the immune activation which leads to disease. This has led to the concept that the role of the MHC class II genes in autoimmune diseases such as rheumatoid arthritis is to function as a permissive molecular signal, like a "green light" which signals the immune system to proceed with an attack on a particular target. In the case of rheumatoid arthritis, the target is assumed to reside in the synovial lining of the joints.

T-cells are derived from the thymus and accordingly they are called T-cells. They circulate freely through the blood and lymphatic vessels of the body, and so are able to detect and react against foreign invaders, i.e., viruses, allergens, tumors and autoantigens. Despite their uniform morphology under microscope, T-cells consist of a heterogeneous population of cells with several distinct functional subsets including helpers, suppressors and killers.

Through a recognition system called the T-cell antigen receptor (TCR), T-cells are able to detect the presence of invading pathogens and direct release of multiple,

distinct T-cell lymphokines called T-cell factors, which instruct B lymphocytes to initiate or suppress antibody production, and regulate the white blood system in producing more phagocytes and other white cells to neutralize the pathogens, and destroy tumor cells and virally infected cells. Thus, the detection and binding of pathogens by T-cells is linked to the triggering of T-cell factor release and to the cascade of host defense actions initiated by these factors.

It is thought that T-cells are activated in physiologic situations through their T-cell antigen (Ag) receptors (TCRs). These are believed to bind to antigenic peptides held in the groove of MHC molecules. The Ag-MHC complex is formed on antigen presenting cells (APCs) following internalization and processing of the Ag into a form that can associate with MHC molecules. Both antigenic peptide and MHC molecule are required for T-cell activation. Together they form a trimolecular complex which is somewhat unique in receptor biology. Most ligand-receptor or receptor-receptor interactions are bimolecular. The trimolecular nature of the TCR-Ag-MHC complex has made the interactions involved particularly difficult to dissect.

Several recent studies have focused on characterizing the interactions between antigenic peptides and MHC molecules. Direct binding of antigenic peptides to MHC molecules has been convincingly demonstrated by several groups. S. Buus et al., "Interaction between a 'processed' ovalbumin peptide and Ia molecules," *Proc. Natl. Acad. Sci. USA* 83:3968 (1986); S. Buus et al., "The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides," *Science* 235:1353-1358 (1987); S. Buus et al., "Isolation and characterization of antigen-Ia complexes involved in T-cell recognition," *Cell* 47:1071-1077 (1986); B.P. Babbitt et al., "Antigenic competition at the level of peptide-Ia binding," *Proc. Natl. Acad. Sci. USA* 83:4509-4513 (1986); J.D. Ashwell et al., "T-cell recognition of antigen and Ia molecules as a ternary complex," *Nature*, 320:176-178 (1986); T.G. Gullet et

al., "Immunological self, non-self discrimination," *Science* 235:865-870 (1987); P.M. Allen et al., "Identification of the T-cell and Ia contact residues of a T-cell antigenic epitope," *Nature* 327:713-715 (1987). The characteristics of this binding include a slow on rate and an exceedingly slow off rate which is hastened by acidic pH similar to that present in endosomal compartments. This implies that the Ag-MHC complex present on the surface of antigen presenting cells is long-lived, allowing presentation of the stable complex to the TCRs of several T-cells.

Binding of antigen to the TCR has been difficult to demonstrate except in some very limited situations. For example, T-cell clones specific for fluorescein + MHC have been established, and these have low affinity binding interactions with fluorescein alone. R.F. Siliciano et al., "Direct evidence for the existence of nominal antigen binding sites on T-cell surface Ti alpha-beta heterodimers of MHC-restricted T-cell clones," *Cell* 47:161-171 (1986). This implies a direct interaction of the TCR with Ag in some instances. This was also implied in studies of T-cell mediated association of antigenic peptides with MHC molecules utilizing fluorescence energy transfer, T.H. Watts et al., "T-cell-mediated association of peptide antigen and major histocompatibility complex protein detected by energy transfer in an evanescent wave-field," *Nature* 320:179-181 (1986). These studies showed evidence for resonance energy transfer from fluorescein-labelled antigenic peptide to Texas-red labelled class II MHC molecules in the presence of T-cell hybridomas specific for that Ag + MHC complex. This suggests the formation of a ternary complex between Ag-MHC-TCR.

In contrast, direct binding of MHC to TCRs has not been established. Studies that have addressed specific

interactions of MHC molecules or MHC-derived peptides with T-cells have all utilized functional read outs such as cellular lysis or cytokine production. J. Schneck et al., "Inhibition of allorecognition by an H-2Kb-derived peptide is evidence for a T-cell binding region on a major histocompatibility complex molecule," *Proc. Natl. Acad. Sci. USA* 86:8516-8520 (1989); W.R. Heath et al., "Mapping of epitopes recognized by alloreactive cytotoxic T lymphocytes using inhibition by MHC peptides," *J. Immunol.* 143:1441-1446 (1989); J. Schneck et al., "Inhibition of allospecific T-cell hybridoma by soluble class I protein and peptides: estimation of the affinity of a T-cell receptor for MHC," *Cell* 56:47-55 (1989a). In one study (J. Schneck et al., *supra* (1989a)), an allospecific class I restricted T-cell hybridoma was utilized to study the functional effects of soluble class I protein and peptides. This hybridoma was specific for H-2K^{b} with weaker reactivity for H-2K^{bm10} and produced IL-2 in response to these stimuli. IL-2 production in response to H-2K^{bm10} was diminished by soluble H-2K^{b} as well as a peptide derived from amino acids 163-174 of H-2K^{b} but not a similar peptide derived from the H-2K^{bm10} sequence. In another study, (J. Schneck et al., *supra* (1989)), this same H-2K^{b}-derived peptide was demonstrated to inhibit lysis of H-2K^{b} target cells by allospecific cytotoxic T lymphocytes (CTLs) derived from several strains including H-2K^{bm1}, H-2K^{bm13}, H-2K^{bm8}, and H-2K^{bm10}. This peptide also blocked lysis of H-2k^{b} targets but not H-2L^{d} targets by a single bulk CTL culture alloreactive for both specificities. However, studies of a similar peptide derived from amino acids 111-122 of the H-2K^{b} molecule revealed another potential explanation for these findings, W.R. Heath et al., *supra* (1989). While this peptide inhibited lysis of H-2K^{b} targets by an alloreactive CTL clone, this CTL clone also recognized the H-2K^{b} 111-122 peptide when presented by syngeneic H-2K^{d} molecules present on the CTL clone. The authors suggested that the H-2K^{b} 111-122 peptide functioned by inducing self-

presentation of the peptide as opposed to a direct interaction with the T-cell receptors.

Structural studies of MHC molecules have been carried out, specifically for class I MHC molecules. The crystal structure of the HLA-A2 molecule revealed that the antigen binding site is comprised of two parallel alpha helices underlaid by an array of anti-parallel beta pleated sheets. This resulted in the formation of an antigen binding groove, which was occupied by unidentified structures in the crystallized HLA molecule. When the potential intermolecular interactions available to such a binding surface are analyzed, (W.V. Williams et al., "The antigen-major histocompatibility complex-T-cell receptor interaction: a structural analysis," *Immunological Res.* 7:339-350 (1988)), the role of antigen within the binding groove in enhancing interaction with the T-cell receptor can be at least two-fold. In one scenario, the TCR has a low affinity for the MHC molecule alone, and the antigen functions chiefly by directly binding the TCR, enhancing the affinity of the TCR for the Ag-MHC complex. In the other scenario, the TCR has a low affinity for the MHC molecule which is due to some strong attractive interactions and some similarly strong repulsive interactions. In this instance antigen functions by reducing repulsive interactions, for example by conformationally altering the orientation of repulsive residues.

Several recent studies have developed molecular models of TCR-Ag-MHC interactions based on functional data. J.S. Danska et al., "The presumptive CDR3 regions of both T-cell receptor alpha and β chains determine the T-cell specificity for myoglobin peptides," *J. Exp. Med.* 172:27-33 (1990); M.M. Davis et al., "A model for T-cell receptor and MHC/peptide interaction," *Adv. Exp. Med. Biol.* 254:13-16 (1989); J.M. Claverie et al., "Implications of a Fab-like structure for the T-cell receptor," *Immunol. Today* 10:10-14 (1989); P.J. Bjorkman et al., "Model for the interaction of T-cell receptors with peptide/MHC complexes," *Cold Spring Harbor Symp. Quant. Biol.* 54:365-373 (1989). These are based

on homology of the TCR with immunoglobulin structures. All predict significant contact of the TCR with the alpha helices of MHC molecules.

SUMMARY OF THE INVENTION

There is provided by this invention a novel method of treating an MHC-linked disease in a mammal suspected of needing such treatment comprising administering to said mammal an effective amount of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen.

Further provided by this invention is a novel method of modulating T-cell response restricted by an MHC in a mammal suspected of needing such modulation comprising contacting said T-cells with an effective amount of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen.

It is believed that peptides and peptide mimetics derivable from the MHC antigen recognition site that bind to T-cell receptors are useful as biologically active, immunomodulatory substances as more precisely detailed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Binding of antibodies to peptides. Antibodies were prepared from ascites or culture supernatant by ammonium sulfate precipitation, dialyzed, and diluted in FACS buffer 1% BSA in PBS with .1% sodium azide. Solid phase radioimmunoassay (RIA) was utilized to study binding as described. In A, binding of different antibodies to

increasing amounts of IA^k_{68-83} peptide is shown. In B, binding of a single dilution (1:10) of 10.2.16 to increasing amounts of peptide is shown. In C, binding to 8 μg/well by increasing amounts of 10.2.16 is shown.

Figure 2. Ability of peptides to inhibit binding of 10.2.16 to IA^k molecules. Antibodies were preincubated with 1 mg/ml (A) or varying amounts (B) of peptides prior to use in FACS analysis for binding to IA^k molecules expressed on RT4.15.HP cells. In A, the Δ mean channel number is shown for cells stained with 10.2.16 versus 15-1-5P. In B, the % decrease in Δ mean channel number is shown for 10.2.16 binding in the presence of increasing amounts of IA^k_{68-83} peptide.

Figure 3. Inhibition of D10.G4 proliferation by IA^k_{68-83} peptide. In A, counts per minute (CPM) incorporated is shown versus increasing amounts of IA^k peptide for specific antigen (conalbumin) and anti-TCR ϵ antibody (2C11). In B, % inhibition of proliferation is shwown for CPM incorporated in the presence of increasing amounts of IA^k_{68-83} peptide.

Figure 4. Antigen presenting cell (APC) dose dependence of IA^k_{68-83} peptide inhibition of D10.G4 proliferation. D10.G4 cells were stimulated with conalbumin and two doses of APCs as described in materials and methods, in the presence of varying amounts of IA^k peptide. % maximal ΔCPM incorporated is shown for increasing doses of peptide. Maximal ΔCPM incorporated with 5×10^5 APCs was approximately 15,000, and with 5×10^4 APCs was approximately 5,000.

Figure 5. Inhibition of anti-clonotype binding by IA^k_{68-83} peptide. D10.G4 were preincubated with IA^k_{68-83} peptide (1 mg/ml) prior to staining with antibodies as noted in materials and methods. Δ mean channel number was calculated by subtracting the mean channel number in the absence of antibody from that in the presence of antibody, and % decrease calculated. The mean ± standard error is shown for two experiments.

Figure 6. Inhibition of anti-clonotype binding by peptide-bovine serum albumin (BSA) conjugates. The protocol

is as noted above, with the exception that peptide-BSA conjugates were used instead of uncoupled IA^k_{68-83} peptide. Conjugates were utilized at 1 mg/ml final concentration.

Figure 7. Binding of IA^k_{68-83} peptide-BSA conjugates to D10.G4 cells. The peptide-BSA conjugates were fluorsceinated as noted in Materials and Methods. Cells were incubated with a 1:10 dilution of fluoroscein isothiocyonate (FITC)-peptide-BSA in FACS buffer for 45 minutes at room temperature, washed twice and analyzed. D10.G4 or 22.D11 cells were incubated with either FITC-1S1 peptide-BSA (left), or with FITC-IA^k_{68-83} peptide-BSA (right). The mean channel number is shown for the different cell lines incubated with the conjugates.

Figure 8. Inhibition of FITC-peptide-BSA binding to cells. (A) Cells were preincubated with 100 μl unfluorsceinated peptide-BSA conjugates at 1 mg/ml for 45 minutes at room temperature. The FITC-IA^k_{68-83} peptide-BSA conjugate was then added for an additional 45 minutes at room temperature, the cells washed twice and analyzed. (B) Cells were preincubated with 100 μl of supraoptimal concentrations of each antibody (undiluted ammonium sulfate cuts) for 45 minutes at room temperature. The FITC-IA^k_{68-83} peptide-BSA conjugate was then added for an additional 45 minutes at room temperature, the cells washed twice and analyzed. For (A) the mean channel number is shown for the different cell lines incubated with the conjugates. The % decrease in mean channel number compared with cells incubated with FITC-IA^k_{68-83} peptide-BSA alone is shown for each condition.

DETAILED DESCRIPTION OF THE INVENTION

Methods of modulating mammalian T-cell response restricted by an MHC and methods of treating an MHC-linked disease in a mammal suspected of requiring such modulation or treatment, are provided by the invention. The methods comprise treating the mammal or contacting the T-cells respectively with an effective amount of a peptide, which peptide has an amino acid sequence substantially corresponding

to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen.

The definition of an "MHC-linked disease" as used herein refers to those mammalian diseases where the relative risk for an individual expressing a particular MHC antigen to develop the disease is at least twice the risk of the population at large. Wherein the relative risk is computed from the following:

$$\text{Relative Risk} = \frac{(\%\ \text{antigen-positive patients})(\%\ \text{antigen-negative controls})}{(\%\ \text{antigen-negative patients})(\%\ \text{antigen-positive controls})}$$

Examples of currenty known or suspected MHC-linked diseases are shown in Table I.

TABLE I

DISEASE	ANTIGEN*	RELATIVE RISK
RHEUMATIC		
Ankylosing spondylitis	B27	87
Reiter's syndrome	B27	37
Acute anterior uveitis	B27	10.3
Reactive arthritis (yersinia, salmonella, gonococcus)	B27	18
Psoriatic arthritis, central	B27	10.7
	Bw38	9.1
Psoriatic arthritis, peripheral	B27	2.0
	Bw38	6.5
Juvenile rheumatoid arthritis	B27	4.5
Juvenile arthritis pauciarticular	DR5	5.2
Rheumatoid arthritis	Dw4/ DR4	6.0
Sjogren syndrome	Dw3	9.7
GASTROINTESTINAL		
Gluten-sensitive enteropathy	DR3	21
Chronic active hepatitis	DR3	6.8
Ulcerative colitis	B5	3.8
HEMATOLOGIC		
Idiopathic hemochromatosis	A3	8.2
	B14	26.7
	A3,B14	90
Pernicious anemia	DR5	5.4

DISEASE	ANTIGEN	RELATIVE RISK
SKIN		
Dermatitis herpetiformis	Dw3	13.5
Psoriasis vulgaris	Cw6	4.8
Psoriasis vulgaris (Japanese)	Cw6	10.7
Pemphigus vulgaris (Jews)	DR4	32
	A10	5.9
Behcet's disease	B5	6.3
		12.7
ENDOCRINE		
Juvenile diabetes mellitus	DR4	5.3
	DR3	2.8
	DR2	0.2
	B1F1	15.0
Graves' disease	B8	3.6
	Dw3	3.7
Graves' disease (Japanese)	Bw35	3.9
Addison's disease	Dw3	10.5
Subacute thryoiditis (de Quervain)	Bw35	13.7
Hashimoto's thyroiditis	DR5	3.2
NEUROLOGIC		
Myasthenia gravis (without thymoma)	B8	4.4
Multiple sclerosis	DR2	3.9
Manic-depressive disorder	Bw16	2.3
Schizophrenia	A28	2.3
RENAL		
Idiopathic membranous glomerulonephritis	DR3	5.7
Goodpasture's syndrome (anti-GBM)	DR2	15.9
Minimal change disease (steroid response)	B12	3.5
Polycystic kidney disease	B5	2.6
INFECTIOUS		
Tuberculoid leprosy (Asians)	B8	6.8
Paralytic polio	Bw16	4.3
Low vs. high response to vaccinia virus	Cw3	12.7

* HLA antigens and diseases, showing the most highly associated antigens in white populations.

Standard methods for determining a mammalian MHC of interest in a tissue are available. More recently, methods

for molecular tissue typing an MHC in a mammal have been demonstrated. Gao, X. et al., "DNA typing for class II HLA antigens with allele-specific or group-specific amplification I typing subsets of HLA-DR4," *J. of Human Immunology* 27:40-50 (1990).

As used herein, the phrase "peptide mimetic" refers to any compound that functionally mimics the peptides described herein. That is, a peptide mimetic must be capable of binding with a T-cell antigen receptor which T-cell antigen receptor recognizes the MHC bound to an antigen, i.e. the T-cell antigen receptor is capable of binding with the MHC-Ag. The T-cell antigen receptor is of the type that specifically binds the MHC-antigen fragment complex.

As used herein the "antigen recognition site" of the MHC refers to that portion of the MHC that is responsible for normal antigen presentation to the T-cell receptor. It is generally believed that the antigen binding site approximates a "groove" formed from two alpha helices lined on the bottom by β pleated sheets as described in Brown, J.H. et al., "A hypothetical model of the foreign antigen binding site of Class II histocompatibility molecules", *Nature*, 332:845-850 (28 April 1988).

Peptides useful in this invention have an amino acid sequence which substantially corresponds to at least a portion of the antigen recognition site. It is only necessary that the peptide or peptide mimetic are capable of binding to the T-cell antigen receptor, which receptor in its unbound state, is capable of binding (recognizing) with an antigen-MHC complex. The amino acid sequence of the peptide will preferably substantially correspond to at least a portion of the alpha helices of the antigen recognition site. Examples of methods to select peptides and peptide mimetics suitable for use in this invention are discussed below.

The vast majority of MHC antigens for the MHC-linked diseases shown in Table I have been characterized, i.e. the amino acid sequence of the MHC has been determined. The known

sequences are published and/or available from a variety of commercial data bases, such as GenBank.

Where the MHC antigen sequence is unknown, procedures are known in the art for determining the sequence of the MHC antigen. Examples of references teaching cloning and sequencing an MHC of interest include Pohea, et al., "Allelic variation in HLA-B and HLA-C sequences and the evolution of the HLA-B alleles", *Immunogenetics* 29, 297-307 (1989); Krangel M.S., "Secretion of HLA-A and -B antigens via an alternative RNA splicing pathway", *J. Exp. Med.* 163:1173-1190 (1986); Weiss et al., "Organization, sequence and expression of the HLA-B27 gene: A molecular approach to analyze HLA and disease associations", *Immunobiology* 170:367-380 (1985); Ausubel et al., *Current Protocols in Molecular Biology* (Ausubel, FM, Brent, R, Kingston, RE, Moore, DD, Seidman, JG, Smith, JA, Struhl, K eds.) Greene Publishing Associates and Wiley-Interscience, John Wiley & Sons, New York, NY (1989); Sambrook, et al., *Molecular Cloning, A Laboratory Manual.* (Sambrook, J, Fritsch, EF, Maniatis, T eds) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) and vanZeeland, et al., "Sequence determination of point mutations at the HPRT locus in mammalian cells following *in vitro* amplification of HPRT cDNA prepared from total cellular RNA," *Current communications in molecular biology, Polymerase Chain Reaction.* (HA Ehrlich, R Gibbs, HH Kazazian Jr., eds.), Cold Spring Harbor Press, CSH, NY, pp. 119-124 (1989).

Briefly, to obtain the MHC antigen sequence, a DNA molecule is synthesized which encodes a partial amino acid sequence of the MHC or which represents the complementary DNA strand to such a DNA molecule which encodes a partial amino acid sequence. This synthetic DNA molecule may then be used to probe for DNA sequence homology in DNA sequences derived from the genomic DNA of the mammal or derived from cDNA copies of mRNA molecules isolated from cells or tissues of a mammal. Generally, DNA molecules of fifteen (15) nucleotides or more are required for unique identification of an homologous DNA,

said number requiring unique determination of at least five (5) amino acids in sequence. The number of different DNA molecules which can encode the determined amino acid sequence may be very large since each amino acid may be encoded for by up to six (6) unique trinucleotide DNA sequences or codons. Therefore, it is impractical to test all possible synthetic DNA probes individually and pools of several such DNA molecules can be used concomitantly as probes. The production of such pools which are referred to as "degenerate" probes is well known in the art. While only one DNA molecule in the probe mixture will have an exact sequence homology to the gene of interest, several of the synthetic DNA molecules in the pool may be capable of uniquely identifying the gene since only a high degree of homology is required. Therefore, successful isolation of the gene of interest may be accomplished with synthetic DNA probe pools which do not contain all possible DNA probe sequences. In fact, a single sequence DNA probe may be produced by including only the DNA codons most frequently utilized by the organism for each amino acid, although, it will be appreciated that this approach is not always successful.

One technique to identify a gene sequence employs the Polymerase Chain Reaction (PCR). *See e.g.*, U.S. Patents 4,683,195 and 4,683,202 which patents are incorporated by reference as if fully set forth herein. Essentially PCR allows the production of a selected DNA sequence when the two terminal portions of the sequence are known. Primers, or oligonucleotide probes, are obtained which correspond to each end of the sequence of interest. Using PCR, the central portion of the DNA sequence is then synthetically produced.

In one such method of employing PCR to obtain the gene which encodes a mammalian MHC gene, RNA is isolated from the mammal and purified. A deoxythymidylate-tailed oligonucleotide is then used as a primer in order to reverse transcribe the RNA into cDNA. A synthetic DNA molecule or mixture of synthetic DNA molecules as in the degenerate probe

described above is then prepared which can encode the amino-terminal amino acid sequence of the MHC protein as previously determined. This DNA mixture is used together with the deoxythymidylate-tailed oligonucleotide to prime a PCR reaction. Because the synthetic DNA mixture used to prime the PCR reaction is specific to the desired mRNA sequence, only the desired cDNA will be effectively amplified. The resultant product represents an amplified cDNA which can be ligated to any of a number of known cloning vectors. Not withstanding this, it will be appreciated that "families" of MHC peptides may exist in mammals which will have similar amino acid sequences and that in such cases, the use of mixed oligonucleotide primer sequences may result in the amplification of one or more of the related cDNAs encoding these related peptides.

Finally, the produced cDNA sequence can be cloned into an appropriate vector using conventional techniques, analyzed and the nucleotide base sequence determined. A direct amino acid translation of these PCR products will reveal that they corresponded to the complete coding sequence for the MHC protein.

To locate the antigen binding site of a sequenced MHC, at least two methods are known to those in the art. One can utilize "sequence alignment" as described in Brown, J.H. et al., "A hypothetical model of the foreign antigen binding site of Class II histocompatibility molecues", *Nature*, 332:845-850 (28 April 1988); or by determining the three-dimensional structure of the HLA molecule crystallographically as described in Bjorkman, P.J., et al., "Structure of the human class I histocompatibility antigen, HLA-A2", *Nature*, 329:506-511, (8 October 1987) and Bjorkman, P.J., et al., "The foreign antigen binding site and T-cell recognition regions of class I histocompatibility antigens", 329:512-518 *Nature*, (8 October 1987). Using such methods, the structural features of the antigen recognition site (or binding groove) by inspection of the structure and the corresponding amino acid sequences are thereby identified.

Conveniently, the sequence alignment method is preferred. Once the MHC antigen sequence is known, the MHC sequence can then be aligned for maximal homology, as taught in Brown et al., with HLA-A2 (or other crystallographically known HLA antigen) sequence. The sequences which correspond to the antigen recognition site are those which comprise the alpha helicies described in Brown et al., *supra* (1988). This are the helices lining the groove, and includes amino acid residues 60-86 and 140-174 of the HLA-A2 allele, and those sequences from other HLA types which align with these sequences as described in Brown et al., *supra* (1988).

Once the antigen recognition site of the MHC of interest is identified, at least a portion of the amino acid sequence of the site can be selected for its suitability for use in the method of the invention. It is expected that peptides substantially corresponding to the alpha helices will be particularly useful. For example, the entire sequence of one helix of the recognition site can be employed for testing (eg. residues 60-86 of HLA-A2). Shorter, overlapping peptides for the entire recognition site can be synthesized for testing (eg. HLA-A2 60-70, 65-75, 70-80, etc.). Regions of particular interest can be synthesized for testing, for example a region in the HLA DR4 β chain (Brown et al., *supra* (1988)) which is associated with rheumatoid arthritis, while the QK residues are invariably absent in non-rheumatoid arthritis associated alleles. Thus, one can select amino acid sequences in regions of the MHC antigen binding site that are suspected to have some relevance to the MHC linked disease and select several peptide analogs focused on this region. Although, not being bound to a particular mechanism of action, it is believed that peptides or peptide mimetics as described herein bind to the TCR and inhibihit the cascade of host defense actions triggered by the formation of the tertiary TCR-Ag-MHC complex.

The peptides useful in the methods of this invention can be prepared synthetically or recombinantly by ways known to those in the art.

Peptides or peptide mimetics suitable for use in this invention, can be screened for their ability to bind with a T-cell antigen receptor which T-cell antigen receptor recognizes the MHC bound to an antigen by any method known to those in the art. Standard immunological assays for such binding include: binding by flow microfluorimetry to relevant cell lines; tritiated thymidine incorporation assays or similar assays to measure T-cell proliferation in the presence of the peptides; release of cytokines (such as interleukins) as determined by immunoassay or biological response assays (such as proliferation of cytokine dependent cell lines to the cytokines) in the presence of the peptides; Chromium-51 release or similar assays to measure cytotoxic T-cell activity; direct binding to T-cell receptors by standard ligand-binding assays or by competition; inhibition or stimulation of T-cell activation and/or growth; binding to MHC haplotype-specific antibodies. Other screening methods also are believed useful such as influencing the course of an experimental model of an autoimmune disease *in vivo* or *in vitro*.

It is believed that peptides that are suitable for use in this invention can be as short as two amino acids in length or the alpha helices which is generally expected to be about 60 amino acids in length.

For use as an anti-MHC-linked disease agent, the peptides and peptide mimetics can be formulated into a pharmological composition containing an effective amount of the peptide in a usual nontoxic carrier. See e.g. Gennaro, A., *Remington's Pharmaceutical Sciences*, 17th edition, Mack Publishing Co., Easton, PA (1985). The composition can be administered via a route suited to the form of the composition. Such compositions are, for example, in the form of usual liquid preparations including solution, suspension, emulsion and the like, which are generally given orally, intravenously, subcutaneously, intramuscularly or topically. The composition can also be provided as a dry preparation

which can be reconstructed to a liquid for use by addition of a suitable liquid carrier.

It is expected that the amount of the composition to be administered will vary with the age and sex of the patient, the type and severity of the MHC-linked disease, etc. An effective amount of the peptide or peptide mimetic is that amount capable of treating an MHC-linked disease or that amount capable of modulating T-cell response to an MHC in an animal. It is expected that the composition will be administered at doses of about 0.01 to about 5000 mg/kg/day, calculated as protein, preferably in divided doses.

EXAMPLES

Materials and Methods

Peptides: All peptides were synthesized by solid-phase methods, as previously described. W. Williams et al., "Sequences of the cell-attachment sites of reovirus type 3 and its anti-idiotypic/antireceptor antibody: Modelling of their three-dimensional structures," *Proc Natl Acad Sci USA* 85:6488-6492 (1988a); W.V. Williams et al., "Immune response to a molecularly defined internal image idiotope," *J. Immunol.* 142:4392-4400 (1989). Peptides were purified by passage over sephadex G25 columns, or by HPLC on a TSK 3000 column (Waters) in 50% acetonitrile 50% water in an isocractic run. Peptides were lyophilized prior to use. For cell culture, all peptides were sterilized by irradiation with 10,000 rads (Cobalt source) prior to use. Peptides utilized are shown in Table 1.

For coupling to BSA, peptides were resuspended in 0.1 M $NaHCO_3$ at 6 mg/ml with BSA at 6 mg/ml in 0.1% gluteraldehyde, and stirred overnight exposed to air at 23°C. The peptide-BSA conjugates were dialyzed against three changes of distilled water and lyophilized prior to use.

Peptide-BSA conjugates were fluorsceinated as follows. Fluorscein isothiocyanate (FITC) (Sigma, St. Louis, MO), was dissolved at 1 mg/ml in 0.1 M Na_2CO_3. To this solution lyophilized peptide-BSA conjugate was added at a final concentration of 4 mg/ml. The solution was stirred at 4°C overnight and dialyzed against phosphate buffered saline (PBS) prior to use.

Mice: AKR female mice aged 6-8 weeks were obtained from the National Cancer Institute (Bethesda, MD) and were maintained in accordance with the National Institutes of Health and University of Pennsylvania guidelines.

Cell Culture and Media: D10.G4 cells were obtained from The American Type Culture Collection (ATCC) and grown in RPMI 1640 with added penicillin/streptomycin, L-glutamine, non-essential amino acids, sodium pyruvate, $5x10^{-5}$ M β-mercaptoethanol, (all from GIBCO) and 10% fetal calf serum (FCS) (Hyclone).

Conalbumin was purchased from Sigma (St. Louis, MO). Cells were passaged at $5x10^4$/ml with antigen presenting cells (APCs) (2500 R irradiated AKR spleen cells) at $5x10^5$/ml and conalbumin at 200 μg/ml. Alternatively, cells were passaged in 10% rat spleen cell concanavalin A supernatant weekly. This did not change the antigen responsiveness or antigen receptor expression of the clones, as assessed by proliferation and FACS respectively. 22.D11 cells (murine helper T-cell hybridoma specific for pigeon cytochrome C + I-E^K) were obtained from Yvonne Paterson, and grown in Dulbecco's modified Eagle's media (DMEM) with 10% FCS as described, F.R. Carbone et al., "A new T helper cell specificity within the pigeon cytochrome c determinant 95-104," *Eur. J. Immunol.* 17:897-899 (1987).

Murine L cells expressing the IA^K molecule (RT4.15.HP), J. McCluskey et al., "Cell surface expression of the amino-terminal domain of A kappa alpha. Recognition of an isolated MHC antigenic structure by allospecific T-cells but not alloantibodies," *J. Immunol.* 140:2081-2089 (1988) were kindly provided by Ron Germain (National Institutes of Health), and grown in DMEM 10% FCS with added G418 at recommended concentrations. The cells were resuspended by incubation with Versene (GIBCO, Grand Island Biological Co.), spun and washed prior to use.

Antibodies: The following monoclonal antibodies were utilized: 15-1-5P anti-H-$2K^KD^K$ (murine lgG2b) and 10.2.16 anti-IA^K (murine lgG2b) (both from the American Type Culture Collection, Rockville, MD (ATCC); 3D3 anti-D10.G4 clonotype (murine lgG1), J. Kaye et al., "Both a monoclonal antibody and antisera specific for determinants unique to individual to cloned helper T-cell lines can substitute for antigen and antigen-presenting cells in the activation of T-cells," *J. Exp. Med.* 158:836-856 (1983); J.M. Rojo et al., "The biologic activity of anti-T-cell receptor V region monoclonal antibodies is determined by the epitope recognized," *J. Immunol.* 140:1081-1088 (1989) and C193.5 (C. Janeway, personal communication) (both kindly provided by Dr. Charles Janeway,

Yale University, New Haven, CT); 500A2, W.L. Havran et al., "Expression and function of the CD3-antigen receptor on murine CD4+CD8+thymocytes," *Nature* 330:170-173 (1988) and 145-2C11, P. Leo et al., "Identification of monoclonal antibodies specific for the T-cell receptor complex by Fc receptor mediated CTL lysis," *J. Immunol.* 137:3874-3880 (1986) (Hamster anti-mouse TCR ϵ chain from J. Allison and Jeffrey Bluestone, respectively). Hybridomas were grown in culture media and supernatants filter sterilized prior to use. Some antibodies were further subjected to ammonium sulfate precipitation and dialysis against phosphate buffered saline (PBS) prior to filter sterilization and use, W. Williams et al., *supra* (1988a).

Radioimmunoassay: This was as previously described, W.V. Williams et al., *supra* (1989). Briefly, Peptides were suspended in distilled water at varying concentrations and 50 μl/well evaporated onto 96 well V bottom plates (Dynatech Labs). The wells were washed in PBS, blocked with FACS buffer (1% BSA in PBS with 0.1% sodium azide), and antibodies added at varying dilutions in FACS buffer, 50 μl/well. Antibodies were incubated overnight at 4°C, the wells washed with PBS, and ^{125}I-goat anti-mouse added, 50,000-100,000 counts per minute (CPM) per well, and incubated for >1 hour at 37°C or overnight at 4°C. The wells were washed 10x in tap water, cut out, and counted.

Proliferation Assay: D10.G4 cells (10^4/well) with 2500 rad irradiated AKR spleen cells (see figures for dosages) were cultured for 72 hours with various stimuli. The wells were then pulsed with tritiated thymidine (1μCi/well) for an additional 18 hours, the cells harvested onto glass fiber filters, and counted in a standard liquid scintillation system.

FACS Analysis: This was as previously described, W. Williams et al., *supra* (1988a). Briefly, cells were resuspended at 10^7/ml in FACS buffer and for D10.G4 cells, preincubated with peptides, conjugates or antibodies for 30-60 minutes at 23°C. For IA^k expressing L cells, antibodies were preincubated with

peptides at 23°C for 30-60 minutes prior to addition of cells. Antibodies or FITC-peptide-BSA conjugates and cells were combined, and incubated for 20 minutes at 4°C. The cells were resuspended in 500 ul FACS buffer, spun down and washed prior to addition of secondary antibody (where indicated). FITC goat anti-mouse Ig (Fisher) was added for 20 minutes at 4°C, the cells washed twice, and analyzed as described, W. Williams et al., *supra* (1988). Antibodies were utilized as follows: 15-1-5P, 10.2.16, 3D3, 500A2, and 145-2C11 were prepared as ammonium sulfate cuts of culture supernatant, and were utilized at a 1:50 dilution. C193.5 was utilized as culture supernatant undiluted.

Results

Example 1

Interaction of IA^K peptide with anti-IA^K antibody

The peptides utilized in this study are shown in Table I. The IA^K_{68-83} peptide corresponds to a region predicted to be an alpha helix lining the Ag binding groove of the IA^K molecule. This site contains polymorphic residues potentially involved in recognition by haplotype-specific antibodies directed to the IA^K molecule, J.H. Brown et al., "A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules," *Nature* 332:845-850 (1988). The control peptide (designated 1S1) was designed to have an identical net charge and hydrophobicity as the IA^K peptide. Amino terminal cysteine residues were added to each sequence to allow dimerization of the peptides, thereby increasing their avidity for various receptor structures.

TABLE I

Synthetic Peptides

Designation	Sequence
1S1 SEQ ID NO:1	(Cys) Thr Tyr Arg Tyr Pro Leu Glu Leu Asp Thr Ala Asn Asn Arg
IA^K_{68-83} SEQ ID NO:2	(Cys) Leu Glu Arg Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr

To ascertain the ability of this peptide to fold into a conformation similar to that present in the native molecule, we determined the ability of the anti-IA^K antibody 10.2.16 to bind this peptide on solid phase RIA (Figure 1). As can be seen, a small but definite binding of this antibody to IA^K peptide can be demonstrated in a dose-dependent fashion. A control isotype matched antibody does not significantly bind this peptide, (Figure 1A). Similarly, a control peptide is not significantly bound by 10.2.16 (Figure 1, B&C). This suggests a specific interaction between 10.2.16 and IA^K_{68-83}.

To evaluate if the peptide folds into the appropriate conformation in the liquid phase, the ability of this peptide to inhibit binding of 10.2.16 to murine L fibroblasts expressing the IA^K molecule was determined (Figure 2). The IA^K peptide specifically inhibited 10.2.16 binding without affecting binding of 15-1-5P to $H\text{-}2K^KD^K$ (Figure 2A). This binding inhibition was dose-dependent, while the control peptide had no effect (Figure 2B). This implies that the IA^K_{68-83} peptide binds 10.2.16 in the liquid phase, and is able to mimic the native structure of the IA^K molecule. This also suggests that this peptide can interact with biological macromolecules which also interact directly with the intact IA^K molecule.

Example 2

Inhibition of D10.G4 Activation by IA^K_{68-83}

The ability of IA^K_{68-83} peptide to mimic a portion of the intact IA^K molecule suggests that this peptide might also interact with the TCR on IA^K restricted T lymphocytes. To test this hypothesis, the T-cell clone D10.G4 was utilized, a murine TH_2 clone which responds to IA^K + conalbumin, J. Kaye et al., *supra* (1983); J. Kaye et al., "Growth of a cloned helper T-cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin 1 is required for the expression of receptors for interleukin 2.," *J. Immunol.* 133:1339-1345 (1984). The ability of this peptide to inhibit proliferation of this clone to conalbumin plus IA^K bearing

antigen presenting cells (APCs) was thus assessed. The results are shown in Figure 3. As can be seen, the IA^K peptide produced a dose-dependent inhibition of D10.G4 proliferation in response to conalbumin. This occurs in the micromolar range of concentration. In contrast, proliferation in response to anti-TCR ϵ antibody (145-2C11), which can directly stimulate the cells bypassing the TCR-MHC interaction, is not altered except at very high doses of peptide. Stimulation of D10.G4 in response to other stimuli (anti-TCR Ab 500A2, concanavalin A) was also inhibited by IA^K_{68-83} only at high doses and not to the same extent as inhibition of the conalbumin response, and the IA^K_{68-83} peptide did not inhibit the proliferation of phytohemagglutinin-stimulated human peripheral blood mononuclear cells (data not shown). This suggests that at least some of the inhibition seen is not the consequence of non-specific toxicity due to the peptide, but is cell and stimulus specific.

The effects of this peptide were tested on D10.G4 at several doses of APCs. If the peptide is competing for binding to the TCR, then lower doses should effectively inhibit D10.G4 activation when fewer IA^K molecules are present to activate the TCRs on these cells. Thus, lower doses of IA^K peptide should inhibit D10.G4 activation if a lower concentration of APCs are present to compete for the available TCRs. With less than $5x10^3$ APCs/well, little antigen-specific proliferation was elicited. At $5x10^4$ APCs/well and $5x10^5$ APCs/well, specific proliferation was induced. The effect of increasing amounts of IA^K peptide on D10.G4 proliferation in response to conalbumin and two concentrations of APCs is shown in Figure 4. In the presence of $5x10^4$ APCs/well, as little as 125 μg/ml of IA^K_{68-83} inhibited proliferation by >60%. Inhibition of proliferation was not observed at $5x10^5$ APCs/well until 250 μg/ml of peptide was added. This is consistent with a competition phenomenon between the APCs and the IA^K_{68-83} peptide for interaction with a specific site on the D10.G4 cells.

Example 3

Inhibition of Anti-TCR binding by IA^{K}_{68-83} peptide

Although the above studies suggest an interaction between IA^{K}_{68-83} peptide and the D10.G4 TCR, they do not establish the interaction in a direct way. To further assess this possibility, several antibodies reactive with the D10.G4 TCR were obtained. Initial studies were performed to establish cell staining with these antibodies on FACS analysis. Adequate staining was achieved with two anti-clonotypic antibodies, 3D3 and C193.5, as well as with anti-TCR complex antibodies 145-2C11 and 500A2, and with anti-H2-$K^{K}D^{K}$ antibody 15-1-5P. The ability of IA^{K} peptide to inhibit binding of these antibodies was tested. Slight inhibition was seen for the anti-clonotypes on several assays. The results of two assays are combined in Figure 5. Specific inhibition of anti-TCR binding was weak but reproducible, as evidenced by the ability of this peptide to inhibit binding of both 3D3 and C193.5, while not inhibiting binding of 15-1-5P to H2-$K^{K}D^{K}$ present on the same cells.

The low degree of inhibition produced was potentially due to the low affinity of interaction between IA^{K}_{68-83} peptide and the TCR. To circumvent this problem, a polyvalent derivative was developed by coupling IA^{K}_{68-83} peptide to BSA. A control peptide was similarly coupled, and the ability of these conjugates to inhibit anti-TCR binding was evaluated. A representative experiment is shown in Figure 6. The IA^{K}_{68-83} peptide-BSA conjugate markedly inhibited cell staining by both of the anti-TCR antibodies. In contrast, the control peptide-BSA conjugate had no significant effect. Binding of 15-1-5P to H2-$K^{K}D^{K}$ was inhibited to a lesser extent by the IA^{K}_{68-83} peptide-BSA conjugate (data not shown). This suggests a direct interaction of the IA^{K}_{68-83} peptide with the TCR present on the D10.G4 cells.

Example 4

Binding of IA^{K}_{68-83} peptide-BSA Conjugates to D10.G4 cells

Direct binding of the peptide-BSA conjugates to murine T-cells was next assessed. The peptide-BSA conjugates

were fluorsceinated, and the resultant complexes were utilized to stain D10.G4 cells as well as 22.D11 cells (a murine T-cell hybridoma specific for pigeon cytochrome c in the context of $I\text{-}E^K$). These cell lines were incubated with the different fluorsceinated conjugates, washed and analyzed for fluorscence intensity (Figure 7). While some staining was apparent for both FITC-peptide-BSA conjugates on both cell types, the staining of D10.G4 with FITC-$IA^K_{68\text{-}83}$-BSA (7B) was much higher than either staining of 22.D11 with either conjugate (7C&D) or staining of D10.G4 with FITC-1S1-BSA (7A). The binding of FITC-$IA^K_{68\text{-}83}$-BSA was partially competed by unfluorsceinated $IA^K_{68\text{-}83}$-BSA, but not by 1S1-BSA (Figure 8A). This indicates binding specific for the $IA^K_{68\text{-}83}$-peptide portion of the conjugate. In addition, binding of FITC-$IA^K_{68\text{-}83}$-BSA was also partially inhibited by anti-clonotypic antibodies specific for the D10.G4 T-cell receptor, but not for other components of the CD3 complex present on these cells (Figure 8B). Together, these results suggest that the FITC-$IA^K_{68\text{-}83}$-BSA conjugate bound to the T-cell receptor on the D10.G4 cells, and that this binding was mediated by the $IA^K_{68\text{-}83}$-peptide.

Initially, the ability of the MHC-derived peptide to fold into an appropriate conformation was investigated. The binding of anti-IA^K monoclonal antibody to the $IA^K_{68\text{-}83}$ peptide, and the ability of this peptide to inhibit binding of the antibody to intact IA^K molecules (Figures 1&2) indicated that the peptide could fold into the appropriate conformation for binding.

The $IA^K_{68\text{-}83}$ peptide displayed biological effects in blocking D10.G4 activation in response to conalbumin + IA^K (Figure 3&4). The inhibition of activation seen in these experiments was of interest when compared with results utilizing the peptides without the addition of specific antigen. It is noteworthy that the peptides utilized bore an amino terminal cysteine residue, which should result in the formation of dimeric peptides.

Since TCR cross-linking in the presence of accessory cells often leads to activation, it was possible that the

peptide might activate D10.G4 cells. Indeed in some experiments, an enhancement in proliferation was seen. In one experiment, CPMs incorporated increased from 16,272 ± 7628 in the absence of the IA^{K}_{68-83} peptide to 47,935 ± 6349 in the presence of 500 μg/ml of the IA^{K}_{68-83} peptide. Thus, the inhibition seen in the presence of conalbumin seemed potentially due to competition for binding to the D10.G4 TCR, while in the absence of conalbumin, receptor cross-linking by the peptide may have contributed to activation.

This was supported by the ability of free peptide to inhibit anti-clonotype binding to the TCR of these cells (Figure 5). However, this inhibition was weak and somewhat inconsistent (data not shown). It was reasoned that if the avidity of the peptide was increased, then a more consistent result would be obtained. By utilizing a multivalent conjugate of IA^{K}_{68-83} peptide to BSA, greater inhibition of anti-clonotype binding was seen while the control peptide-BSA conjugate showed little inhibitory effects (Figure 6). Although the IA^{K}_{68-83} peptide-BSA conjugate also displayed some non-specific effects, its inhibition of anti-clonotype binding was generally greater than its inhibition of non-clonotype binding (data not shown). However, further confirmation of the specificity of this conjugates binding was sought.

A fluoresceinated preparation bound significantly higher to D10.G4 cells versus IE^{K} restricted T-cell hybridomas, while a control fluoresceinated peptide-BSA conjugate bound similarly to both cell types (Figure 7). This binding was at least partly specific in that it was diminished by the appropriate peptide-BSA conjugate, as well as by anti-clonotypic antibody (Figure 8).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Weiner, David B.
Williams, William V.

(ii) TITLE OF INVENTION: METHOD OF MODULATING MAMMALIAN T-cell RESPONSE

(iii) NUMBER OF SEQUENCES: 2

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(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Thr Tyr Arg Tyr Pro Leu Glu Leu Asp Thr Ala Asn
1 5 10

Asn Arg

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu Glu Arg Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His
1 5 10

Asn Tyr
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Claims:

1. A method of treating an MHC-linked disease in a mammal suspected of need such treatment comprising administering to said mammal an effective amount of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen.

2. The method of claim 1 wherein said peptide has an amino acid sequence which substantially corresponds to at least a portion of the alpha helices of the antigen recognition site.

3. The method of claim 1 wherein said mammal is a human.

4. A method of modulating T-cell response restricted by an MHC in a mammal suspected of needing such modulation comprising contacting said T-cells with an effective amount of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen.

5. The method of claim 4 wherein said peptide has an amino acid sequence which substantially corresponds to at least a portion of the alpha helices of the antigen recognition site.

6. The method of claim 4 wherein said mammal is a human.

AMENDED CLAIMS

[received by the International Bureau on 19 August 1992 (19.08.92);
original claims 1,2,4 and 5 amended;
new claims 7 and 8 added;
other claims unchanged (2 pages)]

1. [A method of treating an MHC-linked disease in a mammal suspected of need such treatment comprising administering to said mammal an effective amount] The use of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cell antigen receptor which unbound T-cell antigen receptor is capable of recognizing said MHC bound to an antigen, and further provided that said T-cell receptor not be from an alloreactive T-cell, in the preparation of a medicament for treating an MHC-linked disease.

2. The [method] use of claim 1 wherein said peptide has an amino acid sequence which substantially corresponds to at least a portion of the alpha helices of the antigen recognition site.

4. [A method of modulating T-cell response restricted by an MHC in a mammal suspected of needing such modulation comprising contacting said T-cells with an effective amount] The use of a peptide, which peptide has an amino acid sequence substantially corresponding to at least a portion of the antigen recognition site of said MHC, or a peptide mimetic wherein said peptide or peptide mimetic is capable of binding with a T-cellantigen receptor which unbound

T-cell antigen receptor is capable of recognizing said MHC bound to an antigen, and further provided that said T-cell receptor not be from an alloreactive T-cell, in the preparation of a medicament for inhibiting a T-cell response restricted by an MHC gene product.

5. The [method] use of claim 4 wherein said peptide has an amino acid sequence which substantially corresponds to at least a portion of the alpha helices of the antigen recognition site.

7. The use of claim 1 wherein said peptide has an amino acid sequence which substantially corresponds to residues from the α_1 region of said major histocompatibility complex.

8. The use of claim 4 wherein said peptide has an amino acid sequence which substantially corresponds to residues from the α_1 region of said major histocompatibility complex.

STATEMENT UNDER ARTICLE 19

Claims 3 and 6 are withdrawn without prejudice. Claims 1, 2, 4, and 5 have been amended. New claims 7 and 8 have been added.

Claims 1, 2, 4, and 5 have been amended to more particularly define the invention.

Claims 7 and 8 have added further limitations to independent claims 1 and 4. Support for these claims is found in the specification at page 16, lines 5-11.

Applicants request that the application be considered with such amendments and that a speedy and favorable response be forthcoming.

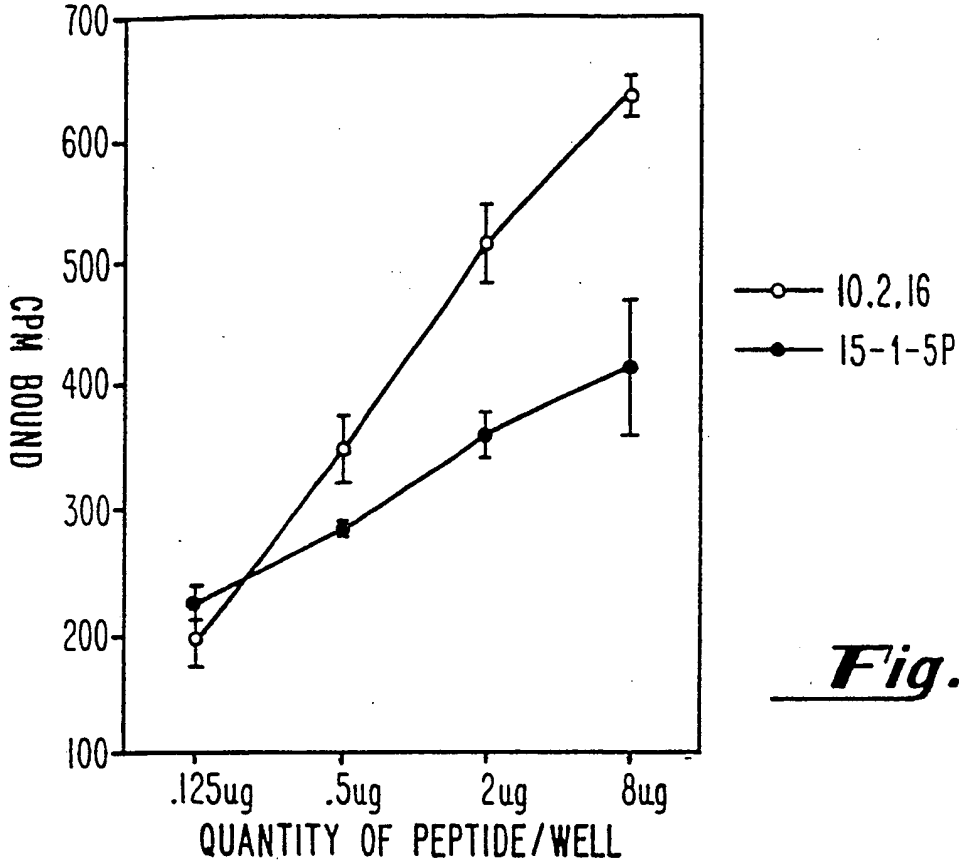

Fig. 1A

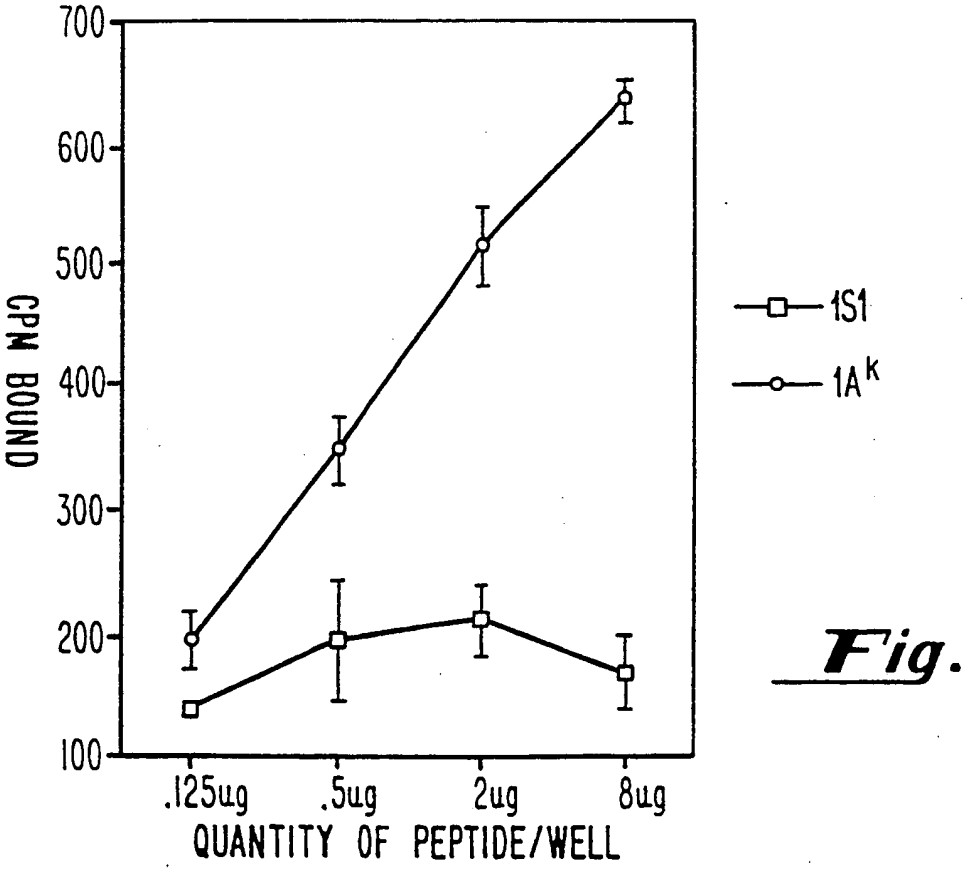

Fig. 1B

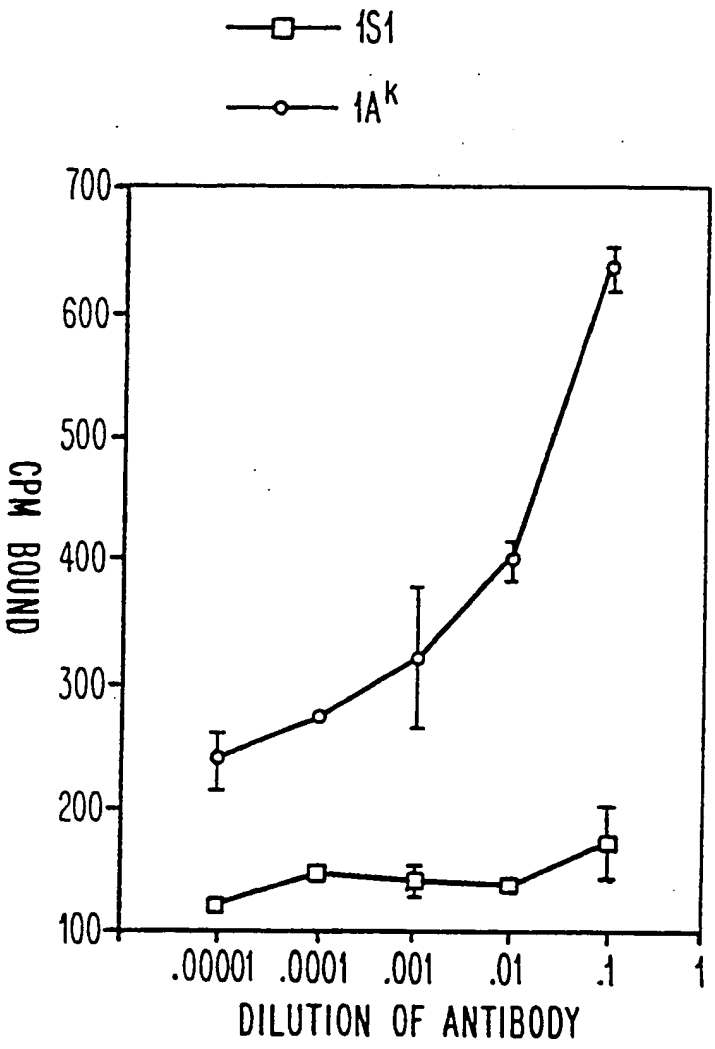

Fig. 1C

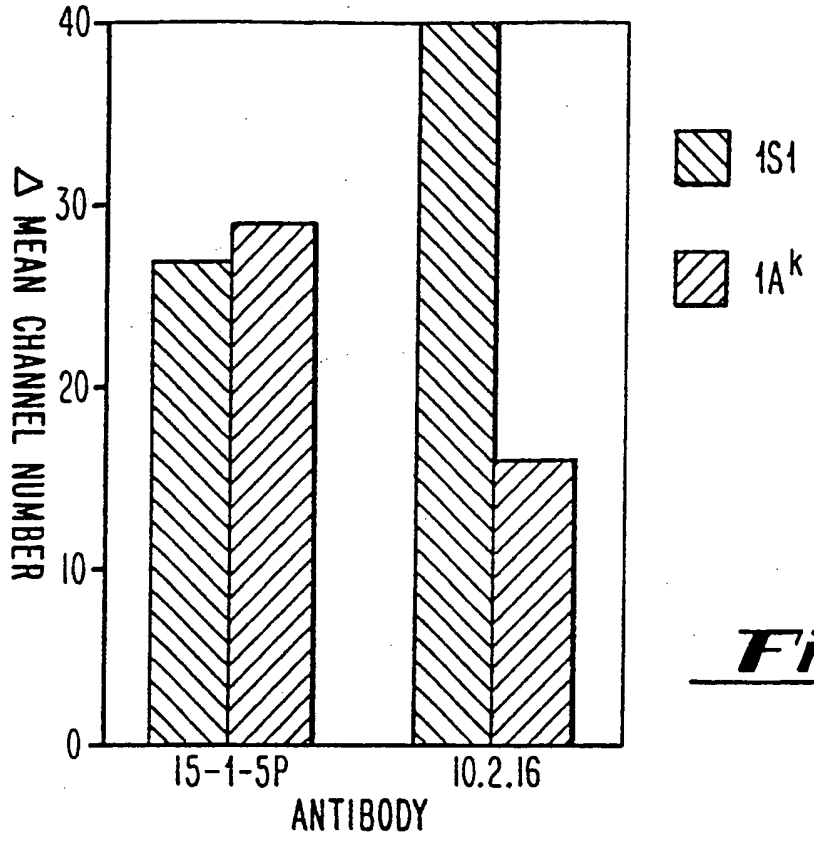

Fig. 2A

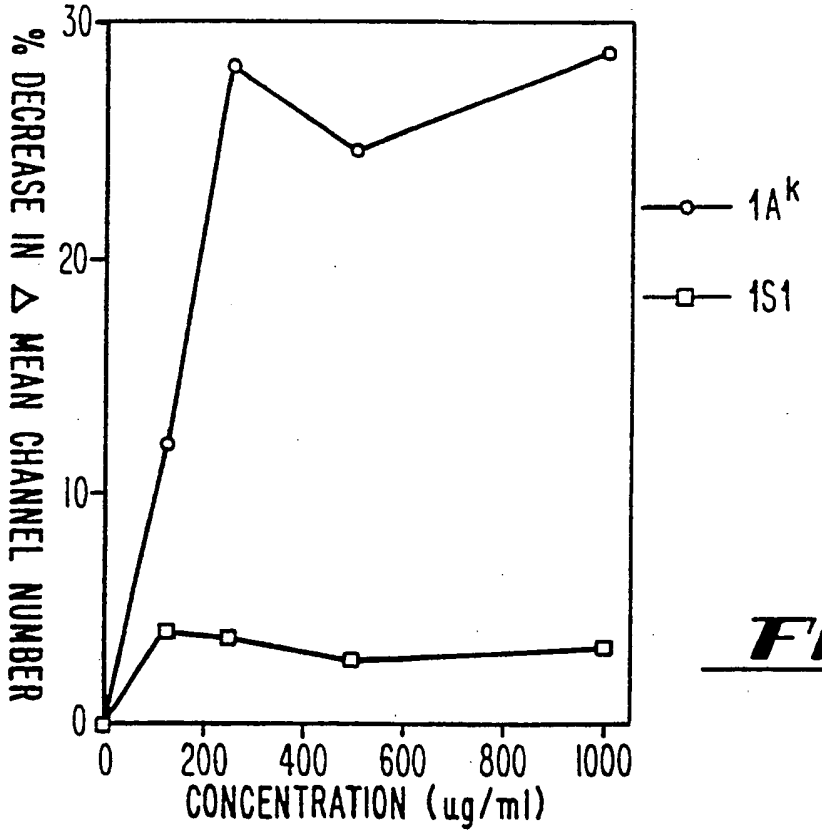

Fig. 2B

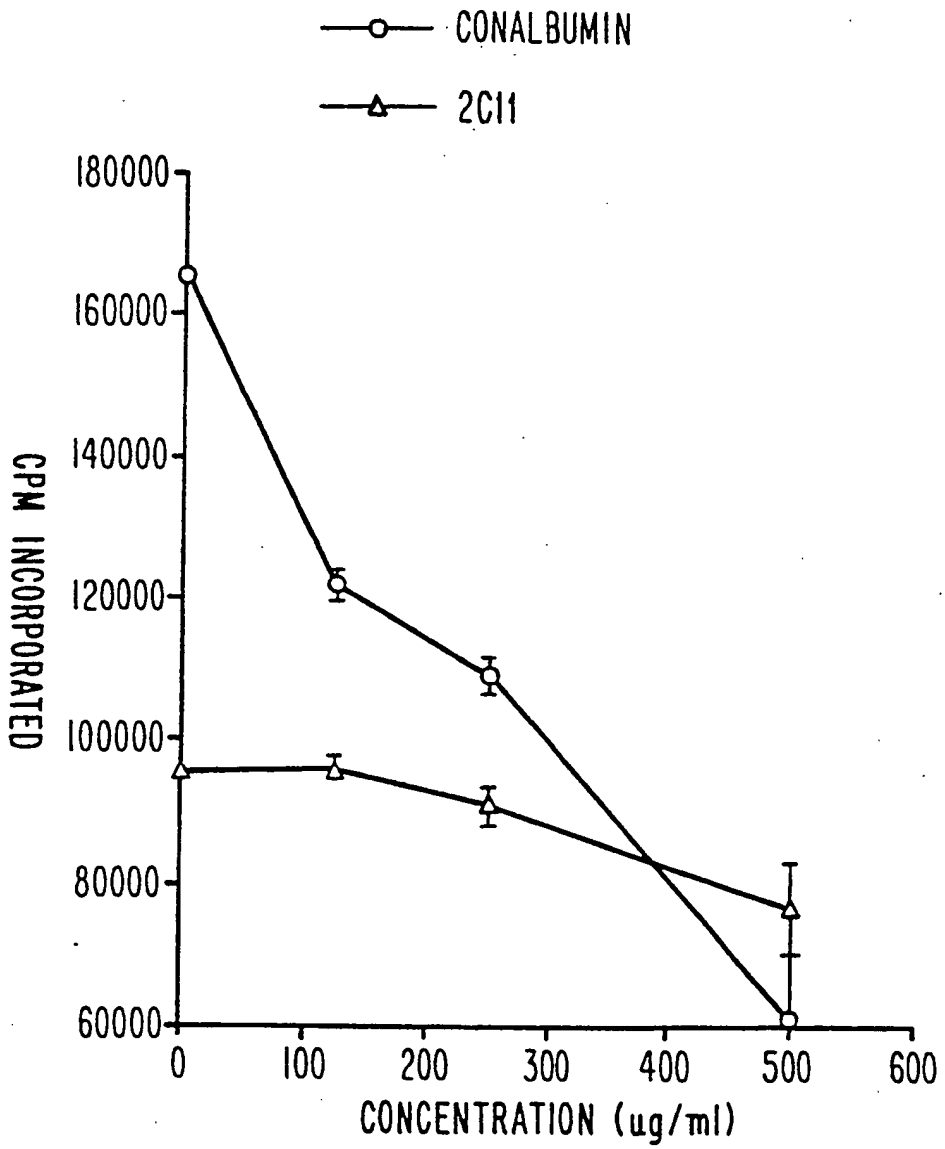

Fig. 3A

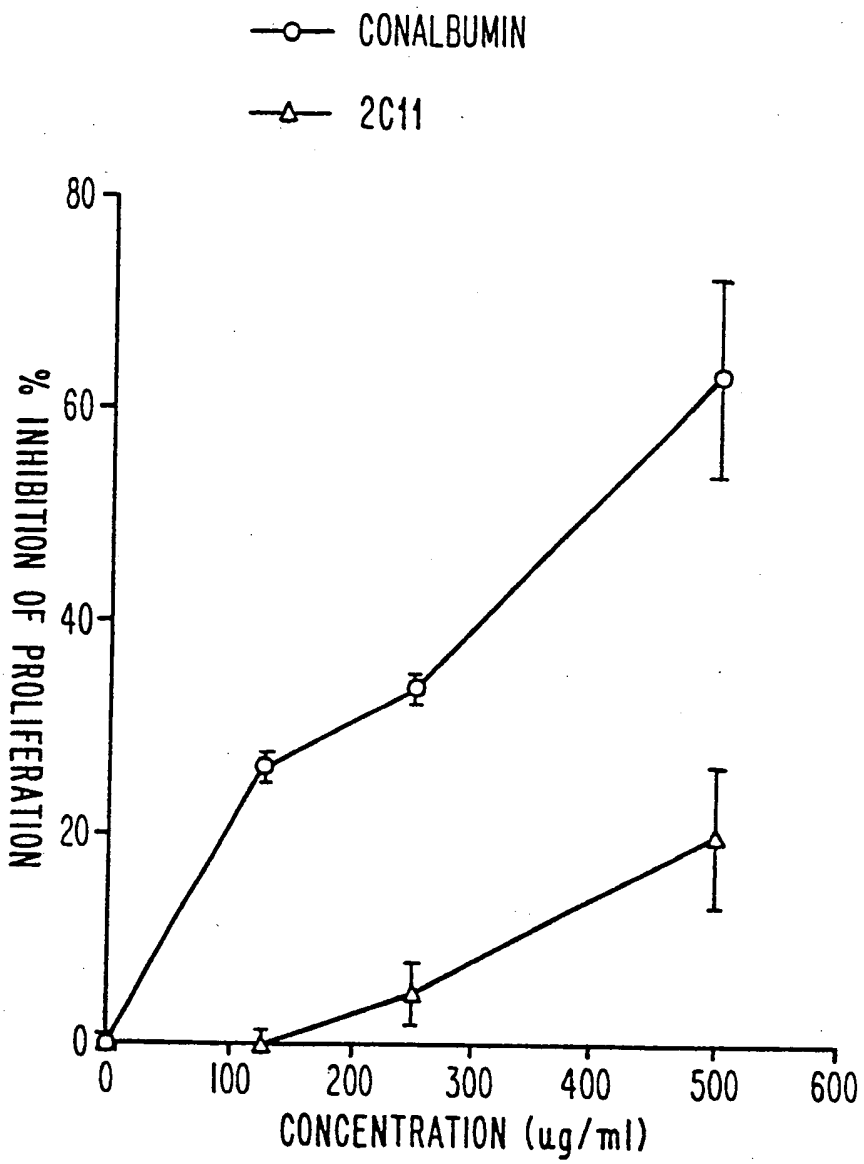

Fig. 3B

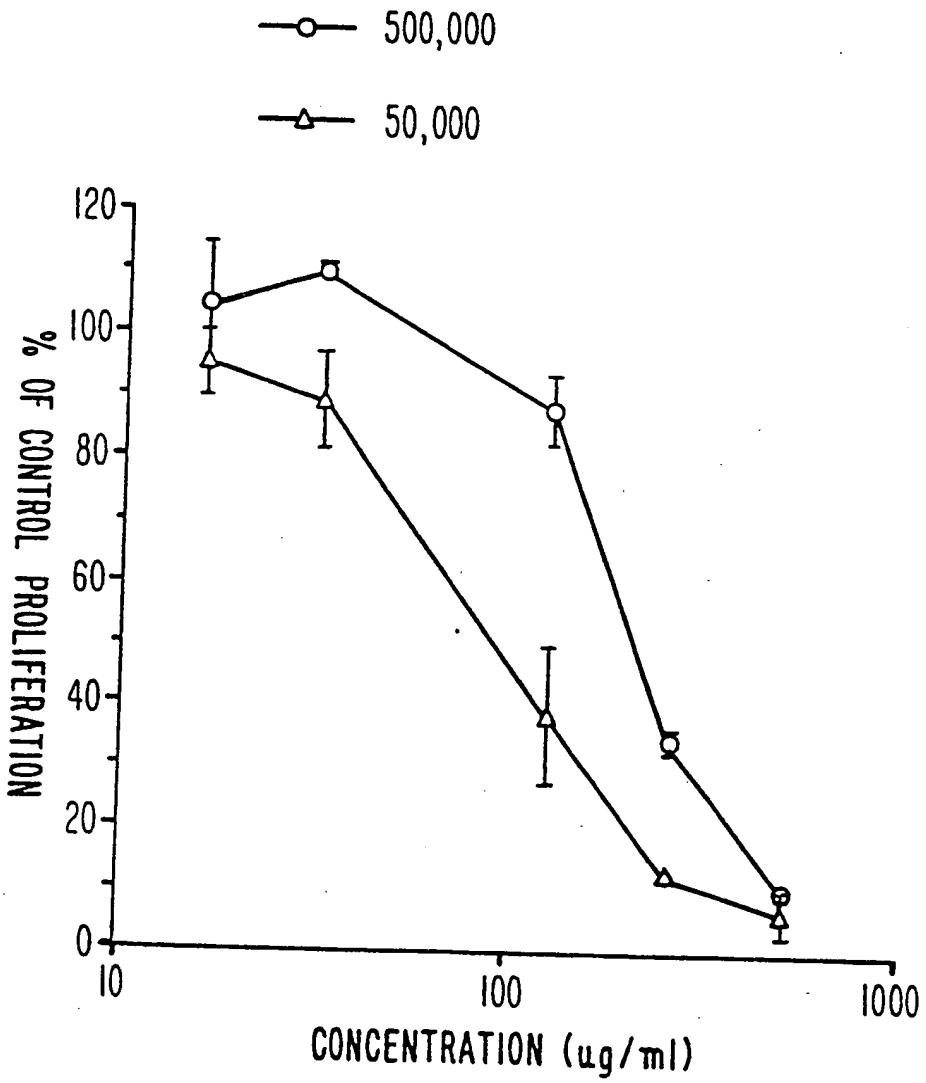
500,000
50,000
120
100
80
60
40
20
0
% OF CONTROL PROLIFERATION
10
100
1000
CONCENTRATION (ug/ml)

Fig. 4

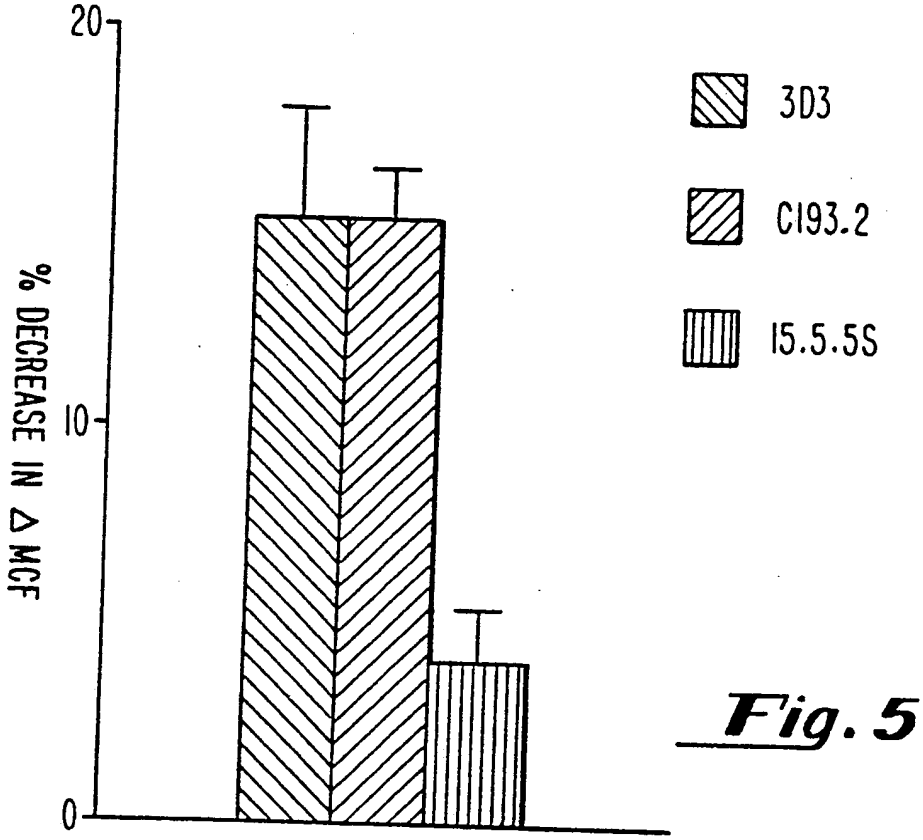
20
10
0
% DECREASE IN Δ MCF
3D3
C193.2
15.5.5S

Fig. 5

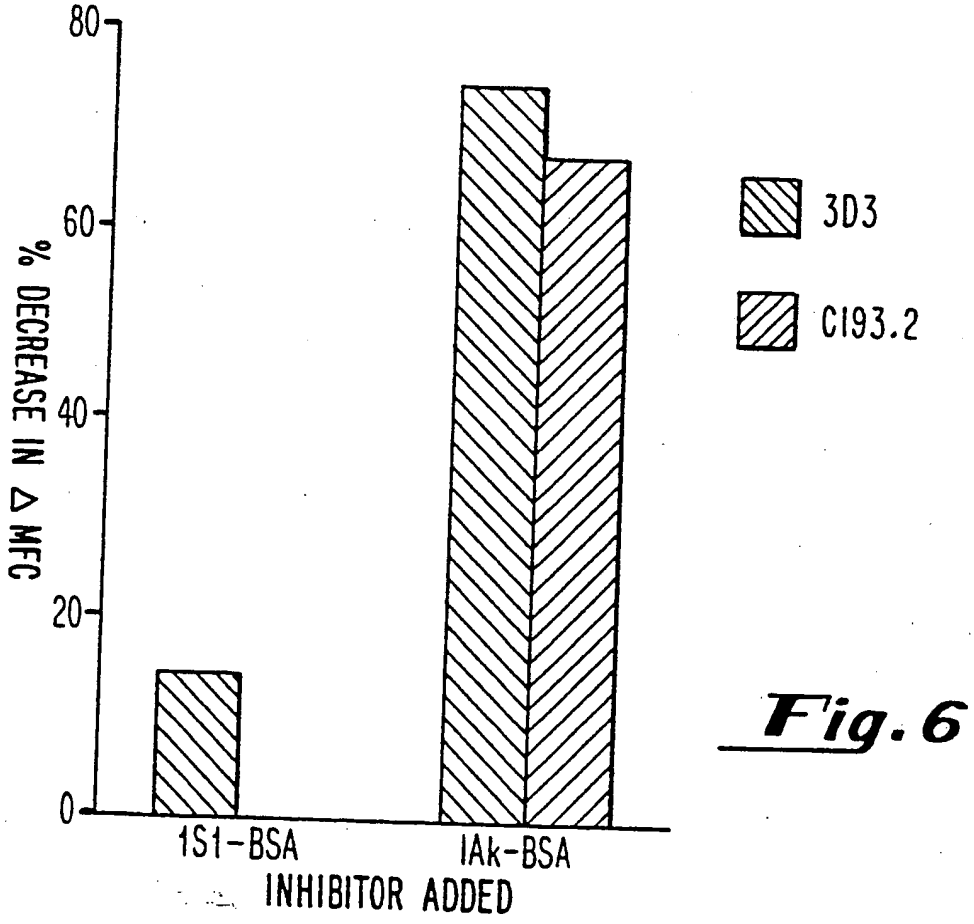
80
60
40
20
0
% DECREASE IN Δ MFC
3D3
C193.2
1S1-BSA
IAk-BSA
INHIBITOR ADDED

Fig. 6

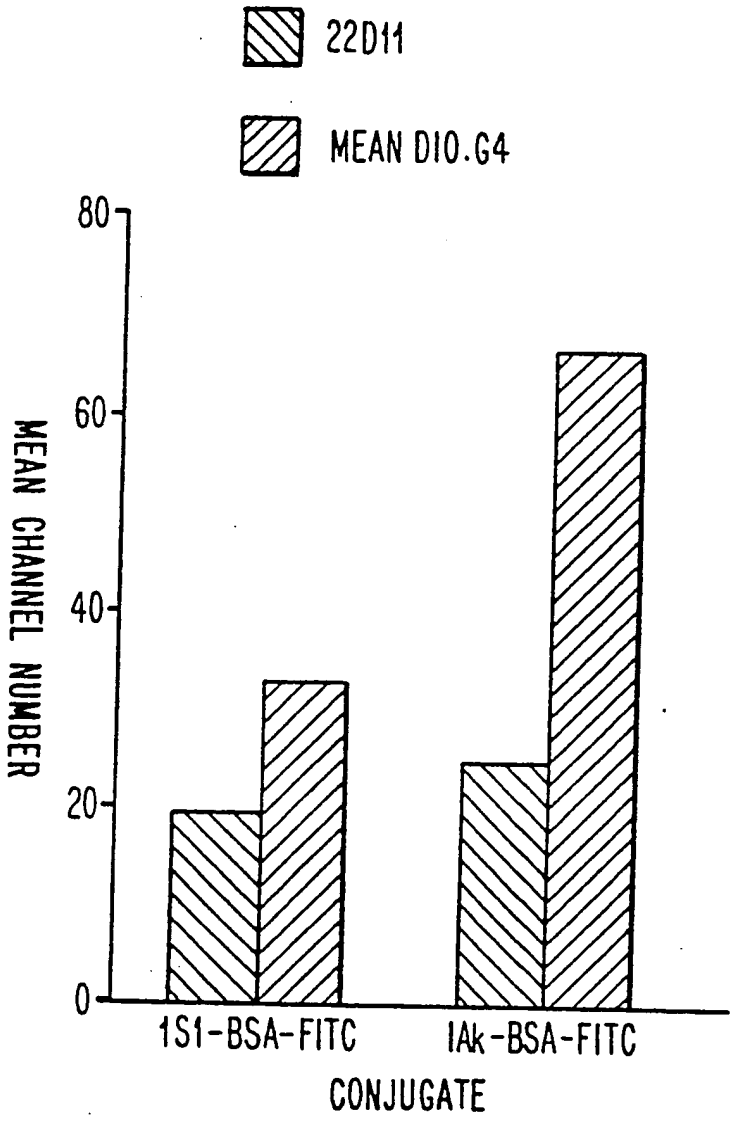
22D11
MEAN D10.G4
80
60
40
20
0
MEAN CHANNEL NUMBER
1S1-BSA-FITC
IAk-BSA-FITC
CONJUGATE

Fig. 7

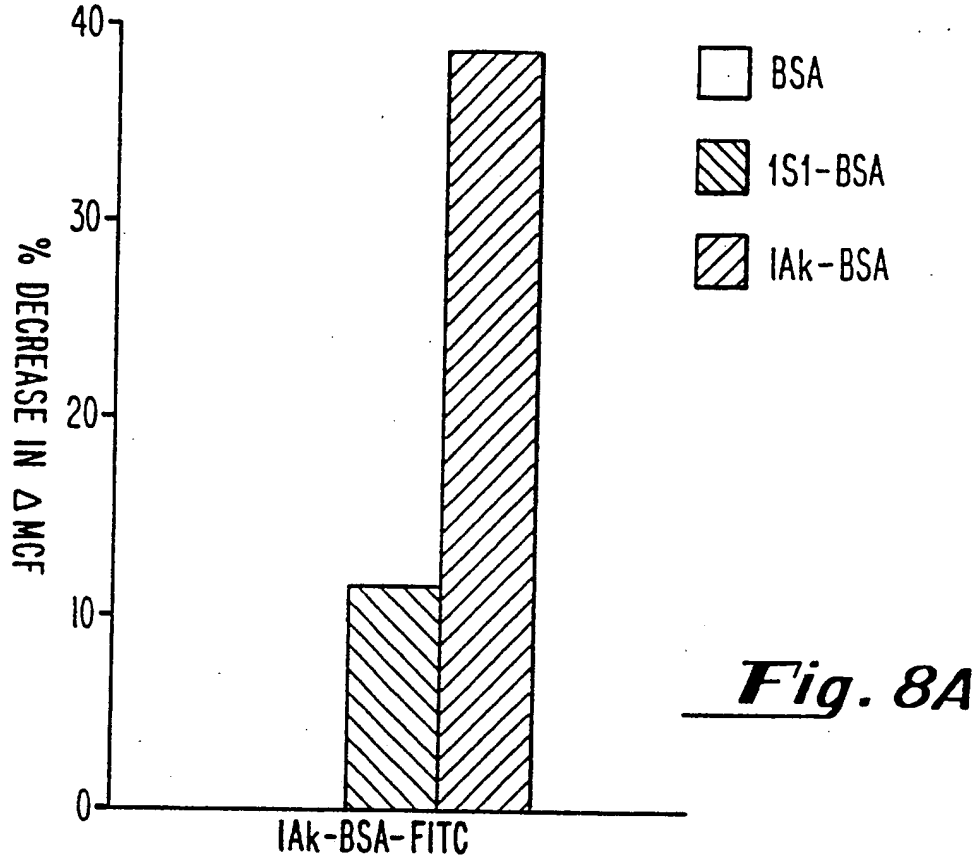
40
30
20
10
0
% DECREASE IN Δ MCF
IAk-BSA-FITC
BSA
1S1-BSA
IAk-BSA

Fig. 8A

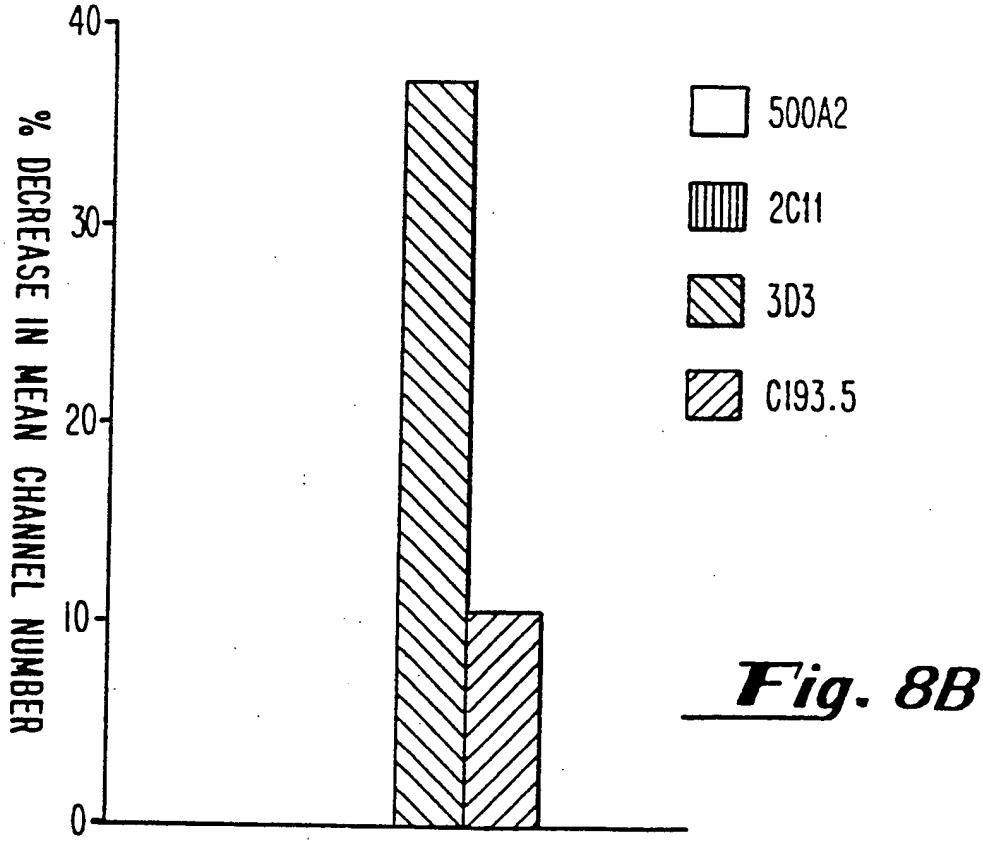
40
30
20
10
0
% DECREASE IN MEAN CHANNEL NUMBER
500A2
2C11
3D3
C193.5

Fig. 8B

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/02419

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 45/05
US CL : 424/85.1

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	424/85.1

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

Dialog, APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y,P	US,A, 5,030,449 (Berzofsky et al) 09 July 1991, see entire document.	1-6
X	Nature, vol. 325, issued 12 February 1987, Parham et al., "Inhibition of Alloreactive cytotoxic T lymphocytes by Peptides from the α_2 Domain of HLA-A2", pages 625-628, see entire document.	1-6
X	Nature, vol 341, issued 12 October 1989, Vandenbark, et al., "Immunization with a Synthetic T-Cell Receptor V-Region Peptide Protects Against Experimental Autoimmune Encephalomyelitis", pages 541-544, see entire document.	1-6
X	Journal of Autoimmunity, vol. 1, issued 1988, Jonker, et al. "Successful Treatment of EAE in Rhesus Monkeys with MHC Class II Specific Monoclonal Antibodies", pages 399-414, see pages 399-414.	1-6

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
10 JUNE 1992	24 JUN 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Deborah Frupe for LYNETTE F. SMITH

Form PCT/ISA/210 (second sheet)(May 1986) &